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Serum Macrophage Colony-Stimulating Factor (M-CSF) Level Is Elevated in Patients With Old Cerebral Infarction Related to Vascular Damage

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We measured the serum levels of macrophage colony-stimulating factor (M-CSF) in 37 patients with an old cerebral infarction who had been surmised to have a damaged vessel wall and who had been in a stable condition for over three months after stroke onset, and those of 41 healthy control subjects. The M-CSF levels in the patients were significantly higher ($P < 0.01$) than those of the controls at 1320.4 ± 410.6 unit/ml and 853.9 ± 180.3 unit/ml, respectively. The plasma levels of von Willebrand factor (vWF) antigen ($P < 0.01$) and thrombomodulin (TM) ($P < 0.05$), as well as those of thrombin-antithrombin III (TAT) complex ($P < 0.05$), prothrombin fragment 1+2 (F1+2) ($P < 0.02$), D-dimer products of crosslinked fibrin degradation products (D-dimer) ($P < 0.01$), and plasmin-antiplasmin (PAP) complex ($P < 0.05$) in the patients were also significantly higher than those in the controls. Significant positive correlations ($P < 0.01$) were found between these parameters and the M-CSF level, but there was no significant correlation between the M-CSF level and the white blood cell count, serum lipids, or blood pressure. Based on these results, we suggest that an increased M-CSF level indicates vascular damage or a thrombotic state in patients with an old cerebral infarction. Am. J. Hematol. 60:185–190, 1999.

Key words: macrophage colony-stimulating factor (M-CSF); cerebral infarction; coagulofibrinolytic activation; thrombotic state

INTRODUCTION

Macrophage colony-stimulating factor (M-CSF), a glycoprotein with a molecular weight of 85,000, is a cytokine that acts as a hematopoietic regulator [1] and is produced by monocytes, vascular endothelial cells, fibroblasts [2], bone marrow stromal cells, ovarian trophoblasts [3], and osteoblasts [4]. This cytokine selectively stimulates the proliferation and differentiation of monocytes and macrophages [1], and may also indirectly stimulate the proliferation of granulocytes and platelets through the generation of other cytokines from monocytes, such as interleukin-6 [5] and granulocyte colony-stimulating factor [6]. Since M-CSF also activates the functions of mature macrophages [7], this cytokine may affect the macrophage functions in atherosclerotic lesions. In an experimental animal model, M-CSF was reported to prevent the progression of atherosclerosis [8]. This preventive effect of M-CSF has been surmised to depend on a lowering of the rate of cholesterol ester accumulation in the arterial wall [8] and on the enhancement of the uptake of acetylated low-density lipoprotein [9]. M-CSF receptor has been reported to be expressed on smooth muscle cells derived from arteriosclerotic lesions, but not on those from normal controls in an animal model [10], suggesting that M-CSF may play an important role in the regulation of atherosclerosis. However, since M-CSF is produced by several types of cells including vascular endothelial cells and macrophages [2], it is surmised that damaged vascular endothelial cells or activated macrophages in atherosclerosis may somehow alter the production of M-CSF.

The serum levels of M-CSF have been reported to be elevated in patients with various diseases including in-

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fectious diseases [11], idiopathic thrombocytopenic purpura [12], aplastic anemia [13], and ovarian cancer [14], as well as in pregnant women [15]. These elevated M-CSF levels may indicate the activated functioning of monocytes-macrophages in these diseases.

In the present study, we measured the serum levels of M-CSF in patients with an old cerebral infarction (as subjects who had vascular damage), and we compared these levels with those of healthy control subjects. Since M-CSF is produced by vascular endothelial cells, we suspected that the serum M-CSF levels in the patients with vascular damage would be altered.

SUBJECTS AND METHODS

Subjects

The subjects were 37 patients with an old cerebral infarction (22 men and 15 women, 72.8 ± 9.4 years, mean ± SD), and 41 healthy volunteers for the normal control group (17 men and 24 women, 69.9 ± 12.1 years). All of the participants were Japanese, and all provided informed consent to participate in the study. The patients had been diagnosed as having a cerebral thrombosis by clinical histories, neurological signs, brain computed tomography, magnetic resonance imaging, or cerebral angiography, and had been in a stable condition for at least three months following the stroke onset. The patients were outpatients of our department. The mean interval between the diagnosis of cerebral infarction and the present study was 18.9 ± 10.6 months. The patients were judged to have relatively mild cases of cerebral infarction, showing almost normal activity in daily life, and they were all in almost the same condition. None of the patients had significant carotid disease or myocardial infarction as judged from the echogram of cervical vessels, and none of the patients was undergoing anticoagulant or fibrinolytic therapy at the time of blood sample collection. The healthy subjects were volunteers who visited the clinic for a health screening during the same period of blood sample collection from the patients. None of the healthy subjects had any signs of or history of serious diseases such as vascular diseases, cancer, or mental or neurological diseases. In the patient group, blood type AB was found in 6 patients (16.2%), A in 13 patients (35.1%), B in 9 patients (24.3%), and O in 9 patients (24.3%), and the distribution in the control group and was not significantly different (12.2%, 26.8%, 34.1%, and 26.8%, respectively).

Methods

Fasting venous blood was obtained and put into tubes free of anticoagulant for serum samples and into tubes with 3.8% sodium citrate (1:9 v/v) for a plasma sample. The serum and plasma were withdrawn following centrifugation at 4°C for 10 min at 1,000×g and then stored at −80°C until assayed.

The serum M-CSF level was measured by an enzyme-linked immunosorbent assay (ELISA) using antihuman M-CSF horse antibody, antihuman M-CSF rabbit antibody and horseradish perioxidase-conjugated anti-rabbit immunoglobulin G goat antibody, as reported previously [11]. The plasma levels of von Willebrand factor (vWF), thrombomodulin (TM), D-dimer products of crosslinked fibrin degradation products (D-dimer), thrombin-antithrombin III (TAT) complex, prothrombin fragment 1+2 (F1+2), and plasmin-antiplasmin (PAP) complex were measured by ELISAs using anti-vWF (Boehringer Mannheim, Mannheim, Germany) for vWF, TM Panacela (Fuji Rebio, Tokyo, Japan) for TM, Enzygnost enzyme immunoassay (EIA) kit (Behringwerke, Marburg, Germany) for TAT, Enzygnost F1+2 (Behringwerke) for F1+2, the Dimertest EIA kit (AGEN Biomedical, Acacia Ridge, Queensland, Australia) for D-dimer, and an α_2-PI complex EIA kit (Teijin, Tokyo) for PAP.

The other laboratory data obtained from each participant were peripheral blood cell counts, serum lipids, serum total protein, transaminases, serum creatinine, and fasting blood sugar levels. Blood pressure was also measured at the time of the blood sample collection.

Statistical Analysis

All results are shown as mean ± SD. The statistical analysis was performed using the Student's *t*-test and linear regression analysis. Differences with a probability value less than 5% were considered significant.

RESULTS

The plasma levels of vWF and TM in the patients with an old cerebral infarction were 191.1 ± 78.0% (mean ± SD), and 3.53 ± 1.12 unit/ml, respectively (Table I). These levels were significantly higher ($P < 0.01$ and $P < 0.05$, respectively) than those in the healthy controls, suggesting that the patient group had vascular damage. The serum level of M-CSF in the patient group (1320.4 ± 410.6 unit/ml) was significantly higher ($P < 0.01$) than that in the control group (853.9 ± 180.3). The plasma levels of TAT, F1+2, D-dimer, and PAP in the patient group were 9.70 ± 4.63 ng/ml, 1.69 ± 0.79 n mol/l, 177.4 ± 144.6 ng/ml, and 0.83 ± 0.27 μg/ml, respectively. These coagulo-fibrinolytic marker levels were significantly higher ($P < 0.05$, $P < 0.02$, $P < 0.01$, and $P < 0.05$, respectively) than those in the control group. The blood pressure, serum lipids, blood cell counts, and other examined laboratory tests did not differ significantly between the patient group and the control group, as shown in Table II.

In the combined group of patients and control subjects

TABLE I. Serum Levels of M-CSF, and Plasma Levels of vWF, TM, TAT, F1+2, D-Dimer and PAP in Patients With an Old Cerebral Infarction and in Healthy Control Subjects†

	Normal range	Cerebral infarction (n = 37)	Healthy control (n = 41)
M-CSF (unit/ml)	610–905	1320.4 ± 410.6*	853.9 ± 180.3
vWF (%)	55–155	191.1 ± 78.0*	140.8 ± 57.0
TM (unit/ml)	0–4.5	3.53 ± 1.12**	2.94 ± 0.73
TAT (ng/ml)	0–0.3	9.70 ± 4.63**	3.61 ± 2.62
F1+2 (n mol/l)	0.4–1.4	1.69 ± 0.79***	1.30 ± 0.39
D-dimer (ng/ml)	0–150	177.4 ± 144.6*	91.3 ± 42.8
PAP (μg/ml)	0–0.8	0.83 ± 0.27**	0.63 ± 0.50

†M-CSF, macrophage colony-stimulating factor; vWF, von Willebrand factor; TM, thrombomodulin; TAT, thrombin-antithrombin III; F1+2, prothrombin fragment 1+2; PAP, plasmin-antiplasmin.
Each value is the mean ± SD.
*$P < 0.01$.
**$P < 0.05$ compared with the healthy control group.
***$P < 0.02$.

TABLE II. Laboratory Findings in Patients With an Old Cerebral Infarction and Healthy Control Subjects*

	Normal range	Cerebral infarction (n = 37)	Healthy control (n = 41)
WBC count ($\times 10^9$/l)	5.0–8.0	5.93 ± 1.29	5.02 ± 1.04
RBC count ($\times 10^9$/l)	3800–4800	3686 ± 1140	4252 ± 336
Hemoglobin (g/dl)	12.0–16.5	15.3 ± 8.0	12.9 ± 1.0
Hematocrit (%)	35–46	42.8 ± 14.6	39.2 ± 2.7
Cholesterol (mg/dl)	130–220	195.8 ± 30.2	207.3 ± 30.1
Triglyceride (mg/dl)	50–150	126.7 ± 56.6	101.7 ± 44.7
HDL cholesterol (mg/dl)	6.5–8.3	54.1 ± 12.3	59.6 ± 12.3
Blood sugar (mg/dl)	70–110	91.2 ± 25.9	95.9 ± 13.6
Total protein (g/dl)	6.5–8.3	6.56 ± 1.62	7.29 ± 0.32
AST (KU)	10–35	21.8 ± 6.9	21.4 ± 5.7
ALT (KU)	5–35	14.1 ± 6.7	15.4 ± 8.2
Serum creatinine (mg/dl)	0.5–1.2	1.1 ± 0.3	0.9 ± 0.2
Uric acid (mg/dl)	2.5–7.0	5.1 ± 1.0	5.0 ± 1.1

*WBC, white blood cells; RBC, red blood cells; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Each value is the mean ± SD.

(n = 78), there were significant ($P < 0.01$) positive correlations between M-CSF and vWF, as well as between M-CSF and TM, as shown in Figures 1 and 2, respectively. When these linear regression analyses were conducted separately in each group, significant positive correlations were also observed in both the patient and control groups (Figs. 3 and 4, respectively). Although there were significant positive correlations between M-CSF and TAT (r = 0.4567, $P < 0.01$), F1+2 (r = 0.3422, $P < 0.01$), D-dimer (r = 0.6361, $P < 0.01$), and PAP (r = 0.5729, $P < 0.01$) in the combined group analysis, there were no significant correlations between M-CSF and the other examined laboratory tests or blood pressure.

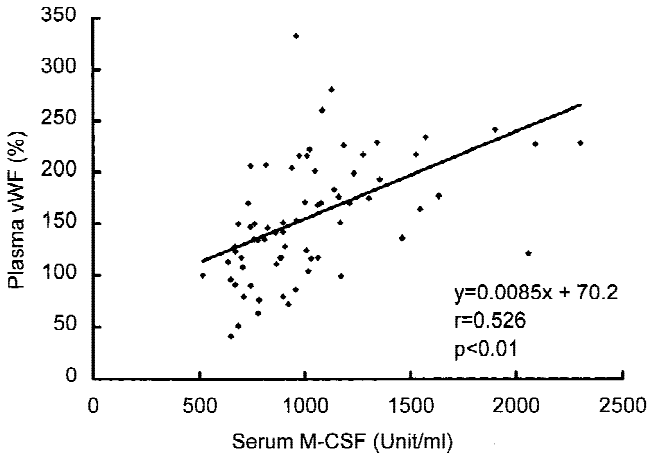

Fig. 1. Correlation between serum M-CSF with plasma vWF levels in the group (n = 78) comprised of both the patients with an old cerebral infarction (n = 37) and the healthy controls (n = 41).

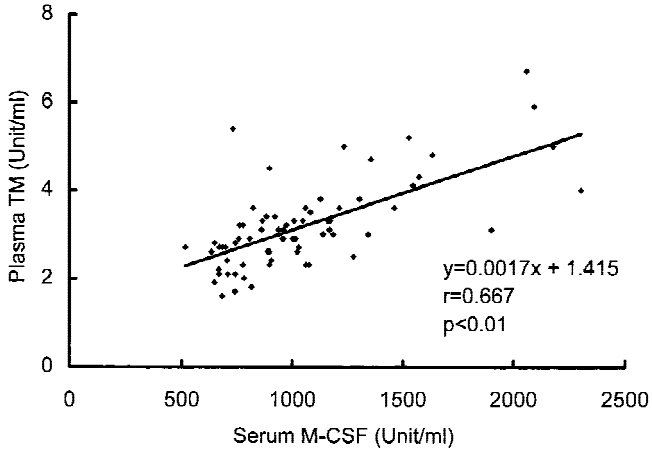

Fig. 2. Correlation between serum M-CSF and plasma TM levels in the group comprised of both the patients with an old cerebral infarction and the healthy controls.

DISCUSSION

We found increased serum M-CSF levels in the patients with an old cerebral infarction. In this patient group, the plasma vWF and TM levels were also increased. Since increased levels of vWF [16] and TM [17] have been acknowledged to indicate an altered vascular endothelial condition, the present patient group was recognized to have some type of systemic vascular damage. Thus, the increased level of M-CSF may be related to the vascular damage in the patients. The significant positive correlations between M-CSF and vWF as well as between M-CSF and TM levels in the linear regression analysis may also support this hypothesis. The plasma level of vWF is reported to vary depending on the ABO blood types [18]. To exclude the effect of ABO blood

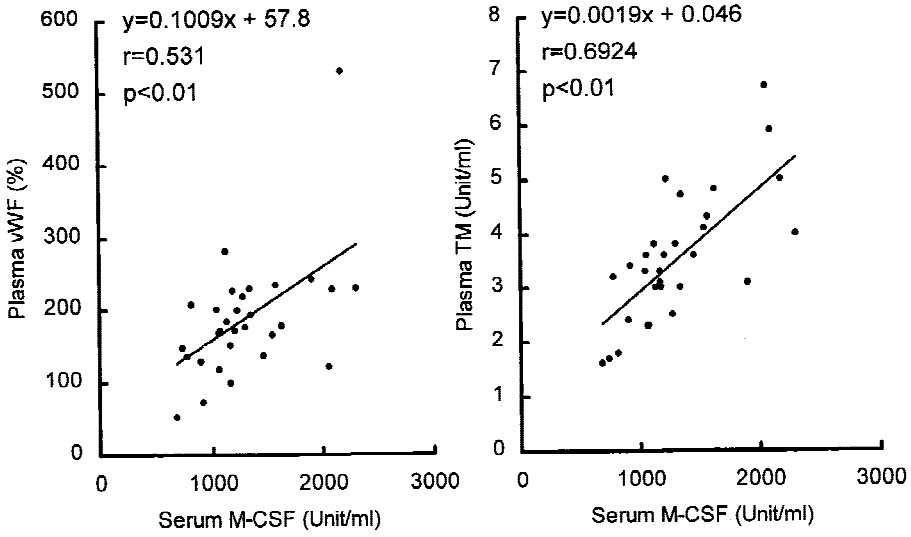

Fig. 3. Correlation between serum M-CSF and plasma vWF levels (left side), as well as serum M-CSF and plasma TM (right side) in the patients with an old cerebral infarction.

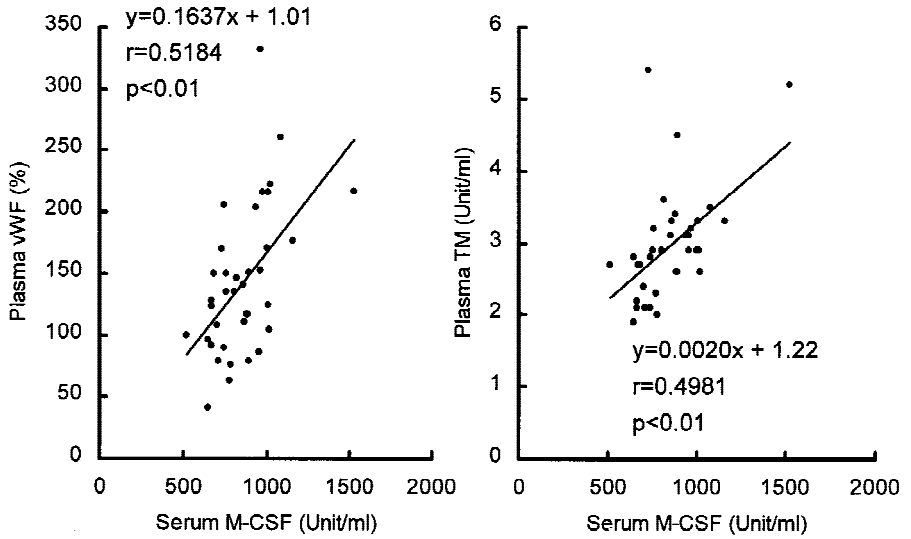

Fig. 4. Correlation between serum M-CSF and plasma vWF levels (left side), as well as serum M-CSF and plasma TM (right side) in the healthy controls (n = 41).

type on the difference of vWF levels between the two present groups, we also investigated the blood types of the participants in each group. No significant difference in blood type distribution was found between the groups.

The markers of the coagulo-fibrinolytic system, i.e., TAT, F1+2, D-dimer, and PAP were also increased in the patients. The plasma level of TAT is a marker of intravascular thrombin generation, and D-dimer and PAP are markers of plasmin generation. F1+2 is a marker of thrombin generation, and is considered to be more specific for thrombin than TAT [19]. We previously reported that the plasma levels of TAT, D-dimer, and PAP were significantly higher in stroke patients than in controls, and that there was no significant difference between the patients with acute and chronic strokes, suggesting that the stroke patients were in a hypercoagulable or thrombotic state caused by a vascular alteration [20]. Acute thromboembolism directly damages the vascular endothelium, which activates the coagulation and fibrinolytic system, an event quickly followed by the release of some vasoactive substances such as adrenaline, noradrenaline, vasopressin, and adenosine diphosphate, which intensify vasoconstriction and platelet aggregability [20]. Therefore, the vascular endogenous response itself may activate the coagulation and fibrinolytic system and affect these marker levels. The elevated levels of these markers may indicate a damaged vascular condition or thrombotic state in the patients with an old cerebral infarction in the present study. The elevated serum M-CSF level in the patient group is also thought to indicate an altered vascular condition or thrombotic state. The significant positive correlations between M-CSF and these coagulofibrinolytic markers may support this possibility.

There were no significant correlations between the serum M-CSF level and the other laboratory parameters, although increased blood pressure values and the levels of serum lipids and fasting blood sugar are acknowledged to be risk factors of thrombosis. Among these

factors, hypertriglyceridemia may correlate with fibrinolytic derangement. In previous studies, since serum triglyceride levels were correlated with plasma tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) levels while serum cholesterol levels were not, hypertriglyceridemia, but not hypercholesterolemia, was suggested to be associated with alterations of the fibrinolytic system [21]. At the time of the present blood sampling, the patient group had received treatment for hypertension, hyperlipidemia, or diabetes mellitus as needed. Thus, there was no significant difference between the patient and control groups in the blood pressure values and the levels of serum lipids and fasting blood sugar. A few of the participants with hypertension were treated with angiotensin-converting enzyme (ACE) inhibitors. Since ACE inhibitors are reported to modify the fibrinolytic system through the reduction of PAI-1 [22], the effect of this medication on the serum M-CSF level should also be investigated. This concept remains to be tested in future studies. Regarding the relationship between the treatment for cerebral infarction and the M-CSF level, we previously reported that anticoagulation therapy using an oral anticoagulant improved the elevated levels of TAT, D-dimer, and PAP in stroke patients [23]. Therefore, it may be possible to modulate the elevated serum M-CSF levels in patients with an old cerebral infarction by anticoagulant therapy. The serum levels of M-CSF may also be applied as a useful index of anticoagulant and other general therapies of cerebral infarction.

In the present study, we chose patients with an old cerebral infarction as representative subjects with a damaged cerebral vessel wall, although it is necessary to distinguish whether the damage of the vessel wall is localized only at the cerebral vessel wall or in the systemic vessels. We also observed a significant positive correlation between the serum M-CSF level and age in healthy control subjects in a preliminary study (unpublished results). A subclinical systemic damaged vessel wall or a thrombotic state is surmised to exist in apparently healthy aged subjects, and the increase of M-CSF might be caused by the systemic damaged vessels. It was reported that the concentration of M-CSF was increased in patients with a myocardial infarction [24]. This finding may indicate that the elevation of the M-CSF level observed in the present patients was caused not only by cerebral vessel damage, but also by a systemic vessel wall alteration including the coronary arteries.

The cause of the increased M-CSF level in the present patients is not clear from the data obtained in this study. As described above, M-CSF may prevent the progression of atherosclerotic lesions [8]. The increase of serum M-CSF may play a role in the negative feedback system in patients with atherosclerotic or thrombotic diseases. The stress caused by chronic stroke or general vascular damage and also the general inflammatory pattern following tissue injury in these patients may modify the M-CSF level. We investigated the M-CSF level of patients with an old cerebral infarction because this cytokine may be related to the progression of atherosclerotic lesions [8]; similar investigations of other cytokines (such as interleukin (IL)-1, tumor necrosis factor-α, IL-10, and IL-6) will contribute to the clarification of the mechanisms underlying the progression of atherosclerosis.

All of the patients and control subjects in this study were Japanese, and the possibility that the data may not be generally applicable to other races should be taken into consideration. The hemostatic functions are reported to be slightly different among different races [25]. However, since the present report is the first attempt to evaluate the relationship between M-CSF and hemostatic molecular markers, the data presented here may still be useful as the basic information for elucidating the clinical significance of M-CSF, as well as blood coagulation and the fibrinolytic system.

In conclusion, elevated serum M-CSF levels were found in patients with an old cerebral infarction who had been surmised to have general vessel wall damage (evaluated using the levels of vWF, TM, TAT, F1+2, D-dimer, and PAP).

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